



# EDGEWOOD CHEMICAL BIOLOGICAL CENTER

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## ASSESSMENT OF THE CAPABILITY OF THE NGDS PROTOTYPE TO REPLACE THE JBAIDS FOR ENVIRONMENTAL SAMPLE ANALYSIS

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## EXECUTIVE SUMMARY

During the week of 21 July 2014, members of the Joint U.S. Forces Korea Portal and Integrated Threat Recognition (JUPITR) Advanced Technology Demonstration (ATD) supported a U.S. Air Force (USAF) team in the evaluation of the BioFire FilmArray system during the USAF exercise, Beverly Midnight 14-03, at Osan Air Base, South Korea. The goal of the USAF team was to conduct an initial evaluation to see if the once-fielded FilmArray system could eventually replace the Joint Biological Agent Identification and Diagnostic System (JBAIDS) for environmental sample analysis.

JUPITR ATD and USAF members analyzed environmental samples using the FilmArray system and JBAIDS in accordance with the USAF Biological Detection Concept of Employment. Exercise participants specifically focused on implementing potential materiel, doctrinal, and procedural changes to reduce the time and costs associated with analyzing samples from aerosol detectors and collectors, such as the Dry Filter Unit (DFU).

The FilmArray system showed enormous potential to rapidly screen samples for a wide range of biological targets. However, known issues with the instrument's hardware, software (version 1.4), and commercially available BioThreat assay pouch caused USAF members to question the instrument's configuration as a potential replacement for the JBAIDS. In light of these observations and lessons learned during the exercise, the JUPITR ATD members made four recommendations.

- **Recommendation 1:** Given that sensitive detection of pooled DFU samples will be critical for the economical implementation of biological field testing, members of the Joint Project Manager (JPM) Guardian's Common Analytical Laboratory System (CALS) program should perform a side-by-side assessment of the FilmArray system and JBAIDS to define the polymerase chain reaction (PCR) performance envelope for the FilmArray system.
- **Recommendation 2:** To ensure the armed services fully adopt the FilmArray system for environmental sample analysis, members of the JPM Medical Countermeasure System's (JPM-MCS) Next Generation Diagnostic System (NGDS) program must derive sensitivity requirements for any government-developed PCR assays from current JBAIDS performance metrics.
- **Recommendation 3:** The JUPITR ATD members should repeat the Beverly Midnight exercises using hardware and software upgrades suggested by BioFire personnel, (1) to evaluate the effectiveness of these vendor-provided upgrades, and (2) to ensure that USAF members are fully satisfied with the FilmArray system as a potential replacement for the JBAIDS for environmental sample analysis.

- **Recommendation 4:** Given the inconsistent performance exhibited by the commercial BioThreat assay pouches, the NGDS program members should institute the BioFire-recommended instrument software and hardware upgrades, assuming that the upgrades are proven effective. They should also consider initiating possible quality-control assay-development measures with BioFire members to ensure that the FilmArray system and its assays are consistent and reliable.

## **PREFACE**

The work described in this report was authorized under contract number W911SR-10-D-0004. The work was started and completed in June 2016.

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# ASSESSMENT ON THE CAPABILITY OF THE NGDS PROTOTYPE TO REPLACE THE JBAIDS FOR ENVIRONMENTAL SAMPLE ANALYSIS

## 1. INTRODUCTION AND BACKGROUND

Beverly Midnight is a set of annual installation-wide exercises designed to test and evaluate the emergency, recovery, and defense operations of the 51st Fighter Wing (the resident tenant unit at Osan Air Base, South Korea) in simulated disaster or emergency environments. During the exercises, members of the Joint U.S. Forces Korea Portal and Integrated Threat Recognition (JUPITR) Advanced Technology Demonstration (ATD) supported the evaluation of the U.S. Air Force (USAF) Biological Detection Concept of Employment (BDCoE). The BDCoE provides USAF installations with a standardized set of service-wide employment concepts that govern the use of biosurveillance assets. These concepts are used to provide installations with a layered approach to defend against biological warfare attacks, with a specific focus on the efficient implementation of environmental detectors and collectors, such as Dry Filter Units (DFUs). The JUPITR ATD members supported the evaluation of the BDCoE by analyzing spiked DFU samples using the Joint Biological Agent Identification and Diagnostic System (JBAIDS) and the BioFire (bioMérieux, Inc., Durham, NC) FilmArray system.



**Figure 1. Participants shown loading samples onto the BioFire FilmArray system during the USAF exercise, Beverly Midnight 14-03.**

The JBAIDS is currently fielded to units across the Department of Defense (DoD). It is a polymerase chain reaction (PCR)-based system that is used to identify a range of biological targets in clinical and environmental samples. The ruggedized JBAIDS includes a sample carousel that enables users to choose the system's multiplex sampling capabilities (i.e., [1] screening one sample for up to five targets using no controls or [2] testing four or more samples for a single target). The JBAIDS requires a minimum of 60 min for sample preparation, which includes 40 min of instrument run time.

The Next Generation Diagnostic System (NGDS) Increment 1 platform will eventually replace all JBAIDSs in the field. The Joint Project Manager (JPM) Medical Countermeasure Systems (MCS) members selected the BioFire FilmArray as the commercial system for the NGDS Inc 1 program (pending an ongoing and upcoming protest). The FilmArray system is a PCR-based instrument that can analyze a single sample during a 60 min run using highly multiplexed assay pouches.

BioFire Diagnostics (Salt Lake City, UT) manufactures a range of pouches that include target assays that are tailored to a specific application or mission. Each pouch requires about 10 min for sample preparation. The BioFire BioThreat assay pouch is used to analyze a

single sample for the presence (or absence) of 17 biological warfare pathogens (21 targets in total).



**Figure 2. The JBAIDS (left) enables operators to choose the system’s multiplex capabilities but requires 60+ min for sample preparation, whereas the FilmArray system (right) uses highly multiplexed assay pouches that require <10 min for sample preparation.**

## **2. ANALYTICAL APPROACH**

During the Beverly Midnight exercises, members of the USAF 51st Medical Group and JUPITR ATD evaluated the BDCoE by analyzing spiked samples that originated from DFUs located across Osan Air Base. There were two main goals for the Beverly Midnight exercises:

- compare the BioFire FilmArray system’s environmental analysis capabilities to those of the JBAIDS during a blind evaluation of spiked DFU filters, and
- evaluate the FilmArray system’s biosurveillance capabilities in accordance with the BDCoE.

During the exercises, bioenvironmental engineering specialists from the 51st Fighter Wing collected DFU filters every 12 h from sites across Osan Air Base. As seen in the Table, some DFU filters were reconstituted with 10 mL of spiked phosphate-buffered saline solution containing one or more of the following inactivated or killed biological agents:

- *Bacillus anthracis* (BA) Ames,
- *Yersinia pestis* (YP) Kim5, or
- Venezuelan equine encephalitis (VEE) Trinidad donkey 1A/B.

The spiking concentrations were based on results from the U.S. Army Edgewood Chemical Biological Center (ECBC) BioSensors Team Test Bed that was conducted in 2012:

- $5 \times 10^3$  cfu/mL for BA,
- $5 \times 10^3$  cfu /mL for YP,
- $1 \times 10^6$  PFU/mL for VEE, and
- blank (unspiked) samples used as controls throughout the exercises.

**Table. DFU Samples Prepared for Analysis**

FilmArray System Pool Number	Number of Pooled DFUs	Concentrated with InnovaPrep?	Spiked Analyte(s)*
P1	5	Y	BA
P2	3	Y	YP, VEE
P3	5	Y	BA, YP, VEE
P4	5	n	BA, YP, VEE
P5	3	n	[blank]
P6	5	n	BA, VEE
P7	5	n	YP
P8	3	n	BA, YP, VEE
P9	5	n	BA
P10	10	n	BA, YP, VEE
P11	3	n	[blank]
P12	10	n	[blank]
P13	3	n	BA, YP, VEE

\*Spiking concentrations =  $5 \times 10^3$  cfu/mL for BA,  $5 \times 10^3$  cfu /mL for YP, and  $1 \times 10^6$  PFU/mL for VEE.

As seen in the Table, various DFU pooling schemes were evaluated using a pre-established pattern of 3, 5, or 10 filters. From each spiked or unspiked reconstituted DFU filter, 3 mL of sample liquid was used for pooling. Some pooled samples included multiple target analytes. In addition, some pooled samples were concentrated using the InnovaPrep (InnovaPrep, LLC, Drexel, MO) concentrating pipette to determine if the device increased the likelihood of detecting low-level concentrations of target analytes.

The pooled samples seen in the Table were analyzed using two instrumentation suites:

- the 51st Medical Group members analyzed pooled samples using the JBAIDS and associated DoD-validated assays, and
- the JUPITR ATD members analyzed samples using the BioFire FilmArray system and commercial BioThreat assay pouch.

At the direction of USAF leadership, the 51st Medical Group did not use the JBAIDS to analyze samples for VEE, although some pooled and spiked samples contained this target analyte.

### **3. OBSERVATIONS AND CONCLUSIONS**

During the Beverly Midnight exercises, the JUPITR ATD and USAF participants recognized the BioFire FilmArray system's capabilities to potentially replace the JBAIDS for environmental sample analysis. The USAF leadership was specifically intrigued by the capabilities of the instrument's multiplexed assay pouches, which were especially effective when evaluating samples that had little to no intelligence of the potential biothreat. USAF members also viewed the assay pouch as a potential force multiplier because of its simplified workflow and integrated sample preparation capabilities, which significantly decreased the training burden associated with the instrument.

However, the JBAIDS outperformed the FilmArray system in terms of assay sensitivity. It should be noted that the JBAIDS assays are not multiplexed, whereas those of the FilmArray system are highly multiplexed. Multiplexing often results in slightly lower sensitivities due to competition for reagents. The difference in multiplexing likely had an impact on the analysis of the samples that were spiked with multiple target analytes during the exercises.

Specific exercise sampling included the following results:

- the FilmArray system correctly identified only five of the 13 pooled samples seen in the Table (a 38.5% true positive rate),
- one false positive occurred on the JBAIDS (sample P11),
- zero false positives occurred on the FilmArray system,
- five false negatives occurred on the FilmArray system, and
- an additional four cases occurred in which the FilmArray system reported positives only for the *Bacillus* species and not the more specific BA.

The exercise participants also experienced software errors on the FilmArray system that were not reported until after the runs were completed (approximately 65–75 min after sample receipt). The software showed that an assay was invalid, and not enough information was available for the user to ensure the success of future runs. JUPITR ATD and USAF staff recognized that these errors were directly linked to a previously identified issue in which the USB and FireWire cables that connected the instrument to the laptop became loose and resulted in a software error.

The JUPITR ATD staff also experienced issues with some BioThreat commercial assay pouches, which failed to draw hydration solution. This issue was seen in half of the pouches from a single box (i.e., three out of six pouches). Because of these issues, no pouches from this box were used for sample analysis.

There were multiple instances during the exercises when the BioFire BioThreat assay pouch meta-analysis identified the presence of one or more BA targets in the sample but issued a summary call of positive for the *Bacillus* species. The instrument's software algorithm did not indicate the presence of BA unless all three assay targets were present. The JUPITR ATD members acknowledged that BioFire users must be on the alert for the false identification of environmental samples as containing BA when, in fact, the samples contain other environmentally ubiquitous near neighbors.

#### **4. RECOMMENDATIONS**

The participants in the Beverly Midnight exercises noticed a distinct difference in the sensitivity of the FilmArray system compared with that of the JBAIDS (the FilmArray system results showed a positive success rate of 38.5% during the exercises, which was significantly lower than that of the JBAIDS, although both instruments were used to analyze the same samples). This was likely because multiple target analytes were spiked into samples, which forced each individual assay on the FilmArray's commercial BioThreat assay pouch to compete for reagents. The ECBC members noticed similar results for the BioThreat pouch during the BioSensors Test Bed evaluation in which assay sensitivity for samples containing only YP CO92 was an order of magnitude lower (better) than samples that contained BA, Vaccinia, and VEE ( $5.0 \times 10^0$  versus  $5.0 \times 10^1$  cfu/mL).

The JUPITR ATD members recognized the labor savings associated with the multiplexed BioThreat pouch and understood that a trade-off existed in terms of assay sensitivity for samples that contained more than one biological target (which could occur when pooling samples from DFUs located across an installation). Therefore, the JUPITR ATD members recommended that additional analyses be performed on the FilmArray system to determine the commercial BioThreat assay pouch performance for DFU samples that contained single and multiple target analytes. These performance results were compared to those for the JBAIDS.

Because the JPM Guardian (JPM-G) Common Analytical Laboratory System (CALS) supports the environmental sample analysis requirements for the NGDS Inc 1 program, the JUPITR ATD members made four recommendations to ensure that the CALS and JUPITR ATD assessments would be performed in parallel.

**Recommendation 1:** Given that sensitive detection of pooled DFU samples will be critical for the economical implementation of biological field testing, the JPM-G CALS program should perform a side-by-side assessment of the FilmArray system and JBAIDS to define the PCR performance envelope for the FilmArray system.

In addition, the JUPITR ATD members recognized that the JPM-MCS team could develop a government off-the-shelf assay pouch for use on the FilmArray system to meet some environmental sample analysis requirements for the CALS program. They recommended that the JPM-MCS leverage these performance characteristics for any government-developed PCR assays, once the performance envelopes for the JBAIDS and FilmArray system were fully characterized.

**Recommendation 2:** To ensure the armed services fully adopt the FilmArray system for environmental sample analysis, the JPM-MCS NGDS program must derive sensitivity requirements for any government-developed PCR assays from current JBAIDS performance metrics.

Exercise participants also experienced hardware and software errors on the FilmArray system. These errors were not reported until after a run was completed, which was approximately 65–75 min after sample receipt (i.e., a previously identified issue with connecting the FireWire data cable caused a known software error). A JUPITR ATD member spoke with BioFire Diagnostics representatives about the FireWire issue. The BioFire team developed a solution and incorporated it into the next iteration of the instrument (version 2.0). The separate FireWire and USB cables that were being used were replaced by a single Ethernet patch cable (Cat5e). The new instrument had onboard data processing capabilities and the capability to store data, if connection with the laptop was interrupted.

The exercise participants also experienced issues with the commercially available BioThreat pouches. During the exercises, the JUPITR ATD staff used three pouches that did not properly draw hydration fluid into the pouch when a sample was introduced. A JUPITR ATD member discussed this issue with someone from BioFire Diagnostics, who surmised that the failures were due to the sample loading stations intermittently pinching the pouch, thereby disallowing hydration of the reagents. BioFire members redesigned the loading station and provided the JUPITR ATD team with the newest iteration.

Because BioFire implemented potential hardware and software solutions, the JUPITR ATD members suggested that the staff should repeat the Beverly Midnight exercises to determine if the upgrades properly address any issues experienced during the exercises.

**Recommendation 3:** The JUPITR ATD should repeat the Beverly Midnight exercises using the hardware and software upgrades suggested by the BioFire team, (1) to evaluate the effectiveness of the vendor-provided upgrades, and (2) to ensure that the USAF is fully satisfied with the FilmArray system as a potential replacement for the JBAIDS for environmental sample analysis.

If the BioFire-recommended software and hardware upgrades are found to increase the performance and consistency of the FilmArray instrument and commercial BioThreat assay pouch, the NGDS program should institute these upgrades for all DoD-fielded FilmArray instruments moving forward. The JUPITR ATD members also recommend that the JPM-MCS members consider an inspection of the BioFire production line for the commercial BioThreat pouch and the other U.S. Food and Drug Administration (FDA)-approved assay pouches, (1) to help identify any potential discrepancies that may exist between FDA- and non-FDA-approved production lines, and (2) to provide recommendations from the DoD to increase the consistency and reliability of the BioThreat pouches.



**Recommendation 4:** Given the inconsistent performance exhibited by the commercial BioThreat assay pouches, the NGDS program should institute the BioFire-recommended instrument software and hardware upgrades, assuming that the upgrades are proven effective. They should also consider initiating possible quality-control assay-development measures with BioFire to ensure that the FilmArray system and its assays are consistent and reliable.

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## ACRONYMS AND ABBREVIATIONS

ATD	Advanced Technology Demonstration
BA	<i>Bacillus anthracis</i>
BDCoE	Biological Detection Concept of Employment
CALS	Common Analytical Laboratory System
DFU	Dry Filter Unit
DoD	Department of Defense
ECBC	U.S. Army Edgewood Chemical Biological Center
FDA	U.S. Food and Drug Administration
Inc.	Increment
JBAIDS	Joint Biological Agent Identification and Diagnostic System
JPM	Joint Project Manager
JPM-G	Joint Project Manager Guardian
JPM-MCS	Joint Project Manager Medical Countermeasure Systems
JUPITR	Joint U.S. Forces Korea Portal and Integrated Threat Recognition
NGDS	Next Generation Diagnostic System
PCR	polymerase chain reaction
USAF	U.S. Air Force
VEE	Venezuelan equine encephalitis
YP	<i>Yersinia pestis</i>



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